American Journal of Pathology, Vol. 168, No. 4, April 2006

DOI: 10.2353/ajpath.2006.050875

Immunopathology and Infectious Diseases

Kupffer Cell-Dependent Hepatitis Occurs during Influenza Infection

Noelle K. Polakos,* Judith C. Cornejo,† Debbie A. Murray,* Kate O. Wright,† John J. Treanor,‡ I. Nicholas Crispe,* David J. Topham,* and Robert H. Pierce†

From the Department of Microbiology and Immunology, David H. Smith Center for Vaccine Biology and Immunology/Aab Institute of Biomedical Sciences, the Department of Pathology and Laboratory Medicine,† and the Department of Medicine Division of Infectious Diseases,‡ University of Rochester Medical Center, Rochester, New York*

Respiratory infections, including influenza in humans, are often accompanied by a hepatitis that is usually mild and self-limiting. The mechanism of this kind of liver damage is not well understood. In the present study, we show that influenza-associated hepatitis occurs due to the formation of inflammatory foci that include apoptotic hepatocytes, antigen-specific CD8$^+$ T cells, and Kupffer cells. Serum aminotransaminase levels were elevated, and both the histological and serum enzyme markers of hepatitis were increased in secondary influenza infection, consistent with a primary role for antigen-specific T cells in the pathogenesis. No virus could be detected in the liver, making this a pure example of "collateral damage" of the liver. Notably, removal of the Kupffer cells prevented the hepatitis. Such hepatic collateral damage may be a general consequence of expanding CD8$^+$ T-cell populations during many extrahepatic viral infections, yielding important implications for liver pathobiology. ***(Am J Pathol 2006, 168:1169–1178; DOI: 10.2353/ajpath.2006.050875)***

Influenza infection in humans has been associated with increased serum aminotransaminase levels, but the incidence of this effect and the etiology underlying the liver damage have not been investigated.[1–3] Immune-mediated hepatitis has been studied in different mouse models; however, most of these experimental systems employ toxins, hepatotrophic pathogens, or systemic antigens to generate immune responses. Although these models of damage are useful for studying the specific immune responses directed toward liver cells, they do not explain hepatic damage during influenza infection of the lung in the absence of viral pathogen in the liver.

In a model in which T-cell receptor-transgenic CD8$^+$ T cells were primed by injection of their specific antigenic peptide, high doses of soluble antigen caused massive CD8$^+$ T-cell expansion,[4,5] accompanied by intrahepatic T-cell accumulation,[4] necro-inflammatory foci, and elevated serum aminotransaminase levels.[5] It could be argued that T-cell localization to these livers is a unique side effect of such nonphysiological priming with antigenic peptide and may reflect the local presentation of peptide in the liver. However, during murine influenza infection, virus-specific CD8$^+$ T cells have been detected in the liver by flow cytometry,[6] indicating that accumulation is not restricted to peptide-activated transgenic cells. Focal lesions have also been noted in these livers,[6] although their cellular composition and pathogenesis have not been addressed.

Influenza productively infects only the epithelial lining of the respiratory tract because the virus requires a unique host trypsin-like enzyme to activate the viral hemagglutinin.[7,8] We have exploited this obligatory localization of virus to the lung as a distinctive approach to explain how an extrahepatic infection can induce bystander hepatitis. This model allows study of interactions between the liver and CD8$^+$ T lymphocytes that have responded to infection at a nonhepatic location.

In C57BL/6 (B6) mice, the antiviral CD8$^+$ response is directed mainly toward two epitopes in the nucleoprotein ($NP_{366-374}$) and polymerase ($PA_{224-232}$) of the virus,[9] which together account for around 30% of the CD8$^+$ T-cell response.[10] These CD8 epitopes are shared by the

Supported by grants from the American Lung Association (RG-075-N) and the National Institutes of Health (NIH) (RO1-AG021970, RO1-AI037554, P30-AG18254, and K08-AI055888) and by NIH/National Research Service Award training grant T32 AI-07285 (to N.K.P.). Histology services were supported in part by the National Institute of Environmental Health Sciences grant ESO 1247.

Accepted for publication December 9, 2005.

Address reprint requests to Robert H. Pierce, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Ave., Box 626, Rochester, NY 14642-8609. E-mail: robert_pierce@urmc.rochester.edu.

serologically distinct influenza A viruses influenza A/HK/x31 (x31) (H3N2) and influenza A/PR/8/34 (PR8 [H1N1]), which induce different antibody responses. Thus, by challenging HKx31-immunized mice with PR8, we were able to study secondary $CD8^+$ T-cell responses in the absence of a protective antibody response, a phenomenon known as hetero-subtypic immunity.[10,11] Here, we use this model to promote the expansion of virus-specific $CD8^+$ T cells, which accumulated in the liver and triggered hepatocyte damage. Through a detailed quantitative and kinetic analysis of this phenomenon, we now provide evidence formally linking influenza-associated liver pathology to the accumulation of influenza-specific $CD8^+$ T cells, and we show that Kupffer cells are essential in this process. Furthermore, the hepatic damage detected in the mouse model by histology and elevated transaminase levels correlated with serological data from a human influenza study. Our findings provide new insight into the etiology of liver damage during influenza infection and offer an explanation for more pronounced liver pathology in other extrahepatic infections like measles and SARS.

Materials and Methods

Experimental Subjects and Viral Infection

Fifteen human male volunteers aged 18 to 45 years were experimentally infected intranasally with 10^7 $TCID_{50}$ influenza A/Kawasaki/86 (H1N1) virus and monitored for 2 weeks for the presence of virus, oral temperature, and symptoms. Virus shedding in nasal wash indicated productive infection. All individuals were seronegative for hepatitis A, B, and C virus, and no medications were taken during the course of infection. Informed consent was obtained from all study subjects, and protocols were approved by the University of Rochester institutional review board. C57BL/6 ($Thy1.2^+$) mice were obtained from Jackson Laboratories (Bar Harbor, ME), whereas $Thy1.1^+$ OT-I, B6.PL ($Thy1.1^+$), TCR $C\alpha^{-/-}$ (a gift from Dr. Deborah Fowell, Department of Microbiology and Immunology, David H. Smith Center for Vaccine Biology and Immunology/Aab Institute of Biomedical Sciences), and DO11.10 mice were bred in-house. NK-deficient mice[12] and major histocompatibility complex (MHC) class II-deficient mice (C57BL/6-$Abb^{-/-}$) were purchased from Taconic (Hudson, NY). All mice were maintained in pathogen-free conditions in accordance with guidelines set forth by the University of Rochester Animal Resources Committee. Animals were infected intranasally with 10^5 EID_{50} of either influenza A/HKx31 (H3N2) (primary) or the serologically distinct influenza A/PR/8/34 (H1N1) (secondary).[13] Some mice received intravenously 200 μl of liposome-encapsulated clodronate[14] (encapsulated by Dr. Nico van Rooijen, Department of Molecular Cell Biology, Vrije Universiteit, Vrije Universiteit Medical Center, Amsterdam, The Netherlands; clodronate provided by Roche Diagnostics GmbH [Mannheim, Germany]) on day 2 after secondary challenge, 72 hours before sacrifice. In OT-I transfer experiments, mice were intranasally infected with 10^4 plaque-forming units
of H1N1 recombinant influenza strain A/WSN-OVA_I, which expresses the SIINFEKL peptide ($OVA_{257–264}$) within the neuraminidase stalk of the virus.[15] TCR $C\alpha^{-/-}$ mice received 500 pfu of the influenza WSN-$OVA_{323–339}$ strain[16] in 30 μl of phosphate-buffered saline (PBS) 1 day after intravenous injection with 2×10^7 splenocytes from DO11.10 mice (which are on a TCR $C\alpha^{-/-}$ background).

Analysis of the Immune Response

Spleen, lymph node, and bronchioalveolar lavage (BAL) cells were prepared as described previously.[13] Livers were perfused, excised, and digested as reported.[5] The digest was diluted with 30 ml of serum-free RPMI and centrifuged twice at $30 \times g$ to remove hepatocytes, followed by a $400 \times g$ spin to pellet lymphocytes. This pellet was resuspended in 26% metrizamide (Sigma-Aldrich, St. Louis, MO) in RPMI, layered beneath serum-free RPMI 1640 medium, and then centrifuged for 20 minutes at 4°C, $1500 \times g$. The lymphocyte interface was collected and washed. Single-cell suspensions were analyzed by flow cytometry (using Pharmingen (San Jose, CA) antibodies unless stated). F_c receptors were blocked with purified α-CD16/α-CD32, and single-cell suspensions were stained with anti-CD8, anti-CD4 (Caltag, Burlingame, CA), anti-CD45.1, anti-Thy1.1, or anti-Thy1.2 antibody, as stated. Influenza-specific cells were detected with phycoerythrin-conjugated H-$2D^b$ MHC class I/$NP_{366–374}$ tetramers[17] (made in collaboration with the Trudeau Institute, Saranac Lake, NY). Data were acquired using a FACScalibur cytometer, and analysis was performed using CellQuest software (both from Becton, Dickinson and Company, San Jose, CA) and FlowJo (Treestar Inc., Ashland, OR). Serum collected by cardiac puncture was frozen at −20°C until analysis of aminotransaminase (aspartate aminotransaminase [AST] and alanine aminotransaminase [ALT]) levels using standard spectrophotometric activity assays (VetCheck, Rochester, NY).

Histology

Formalin-fixed liver sections were embedded in paraffin, and 5-μm sections were cut and stained with hematoxylin and eosin (H&E). Hepatitic foci on H&E-stained sections (n = 3–5 mice/time point) were counted by a pathologist "blind" to the identity of the samples. Counts were normalized to total area of sections counted as previously described,[18] and a Fischer's least significant difference was used for analysis of variance.[19] Alternatively, deparaffinized slides were pretreated with proteinase K (20 μg/ml) and then stained with terminal deoxynucleotide transferase dUTP nick-end labeling reagents (Roche). Slides were incubated with anti-CD3 (DAKO, Carpinteria, CA) and anti-F4/80 antibodies (Caltag) and then secondary Al-

exa 488 goat anti-rabbit antibody (Invitrogen/Molecular Probes, Carlsbad, CA) for CD3 and Alexa 647 goat anti-rat antibody (Molecular Probes) for F4/80. Chromogenic immunohistochemistry was done with biotinylated rabbit anti-rat F4/80 antibody and then streptavidin-HRP antibody (Jackson Laboratories) and developed with AEC$^+$ (DAKO). Hematoxylin was used as the counterstain. Some tissues were snap-frozen in OCT freezing compound and liquid nitrogen. Sections (5 μm) were then fixed with 50:50 methanol:acetone, stained with PE-Thy1.1 or APC-Thy1.2 (Pharmingen) for 1 hour, and counterstained with 100 nmol/L Sytox green (Molecular Probes) for 2 minutes before imaging.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RNA was isolated from 14 flash-frozen mouse lungs and livers using TRIzol reagent (Invitrogen, Carlsbad, CA; manufacturer's protocol). cDNA was made from 1 μg of RNA template using First Strand cDNA Synthesis kit (Roche). The LightCycler PCR/detection system (Roche) was used to amplify and quantify influenza cDNA from each tissue. The LightCycler FastStart DNA Master SYBR Green kit was used as described by the manufacturer. PCR mixtures contained 100 ng of cDNA template, 1× LightCycler reaction buffer, 2.65 mmol/L magnesium chloride, and 0.5 μmol/L sense (5′-GAGCTGGTTCA-GAGTTCCTC-3′) and antisense (5′-TCACAATGAG-GGTCTCCCA-3′) primers to amplify a 110-bp fragment of influenza virus cDNA. Thermal conditions were 55 cycles of 2 seconds at 94°C, 2 seconds at 65°C, and 4 seconds at 72°C. Melting curve analysis was performed from 65 to 95°C, followed by cooling to 40°C. dsDNA product was quantified by monitoring fluorescence of DNA-binding SYBR Green I dye. Standard curve analysis was performed with each run using the 110-bp influenza product cloned into pCR-Blunt (Invitrogen) as template. Level of detection was 2.6 viral copies.

OT-I Cell Transfer and Activation

Spleen and lymph node cells from Thy 1.1$^+$OT-I mice were depleted[20] using rat monoclonal antibodies to CD4 (GK1.5), MHC class II (TIB120), and goat anti-mouse Ig-coated beads (Invitrogen/Dynal, Carlsbad, CA) and goat anti-rat Ig-coated beads. CD8$^+$ T cells (5×10^6; enriched to 96% purity) in 500 μl of PBS were intravenously transferred into allotypically distinct (Thy 1.2$^+$) B6 mice on day −1. For peptide-activated T cells, animals received 25 nmol/L SIINFEKL peptide in PBS intraperitoneally on days 0, 1, and 2 and were then sacrificed on day 5 for histology and flow cytometry. Alternatively, transfer recipients were challenged with the influenza virus WSN-OVA$_I$ on day 0 and then sacrificed on day 9 at the peak of the pneumonic immune response to this virus.[15]

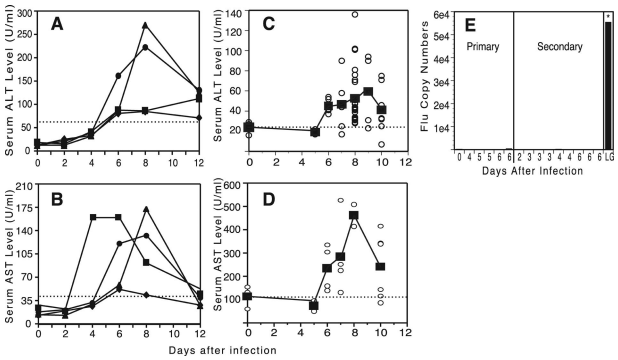

Figure 1. Elevation of serum liver transaminase levels and absence of intrahepatic virus. **A** and **B:** Fifteen healthy male volunteers aged 18 to 45 years were intranasally infected with 10^7 $TCID_{50}$ influenza A/Kawasaki on day 0. Plotted are serum alanine aminotransferase, ALT (**A**), and aspartate aminotransferase, AST (**B**), levels from those subjects exhibiting elevations above the upper limit of normal (**dotted line**) during the course of infection. Those individuals without enzyme elevations are not shown. **C** and **D:** Blood serum was also obtained from mice infected with influenza A/HKx31 and analyzed for ALT (**C**) or AST (**D**). ○, individual mice; ■, means of three or more mice for each time point. **E:** RNA was isolated from liver homogenates or from a day-4 lung (LG) and subjected to quantitative real-time RT-PCR for influenza virus RNA. Copy numbers from uninfected and infected mice were indistinguishable. Detection limit is 2.6 copies of viral genome.

Results

Elevated Serum Aminotransaminase Levels during Influenza Infection Indicate Liver Damage

Elevation of liver transaminase levels is a frequent observation during human influenza infection. However, because clinical attention is on the acute infection in the respiratory tract, the incidence of liver involvement has not been established. To formally test the association of influenza infection with liver injury, we analyzed data from a human influenza study in which volunteers were deliberately infected with influenza A/Kawasaki/86. Four of 15 (26.6%) infected individuals experienced a significant rise in ALT values. In two of these four, peak ALT levels were more than three times the upper limit of normal range (Figure 1A). AST values (Figure 1B) were clinically elevated in 20% of the infected subjects. Notably, these increases in ALT and AST levels were not coincident with pyrexia (which had resolved by day 4), demonstrating that liver damage was not linked to the innate immune response nor to virus replication. A separate study of 54 individuals experimentally infected with influenza B/Yamagata provided similar evidence of influenza-induced liver damage, with liver enzyme abnormalities in 20.3% of these subjects (data not shown). The moderate frequency of liver pathology among human subjects was not unexpected. Most adult humans are likely to have varying levels of pre-existing influenza immunity, and the presence of cross-reactive antibodies could temper the infection and liver injury.

The association between influenza infection and elevated transaminase levels was accentuated in a murine study in which there was no prior exposure to respiratory infection. All animals undergoing primary infection with x31 had elevated ALT (Figure 1C) and AST levels (Figure 1D), peaking on day 8. The high incidence (100%) in

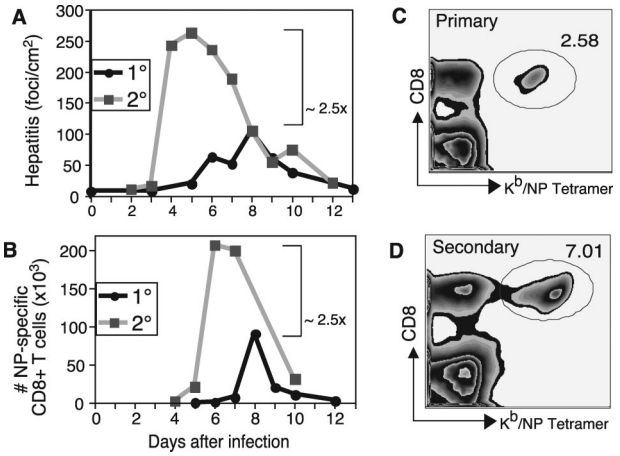

Figure 2. Kinetics and magnitude of hepatitis and virus-specific $CD8^+$ immune response during primary and secondary influenza infections. **A:** Hepatitic foci are plotted after primary infection (black circle) of naïve B6 mice with 10^5 EID_{50} influenza A/HK/x31 or secondary infection (gray square) of HK/x31-immune B6 mice with 10^5 EID_{50} influenza A/PR/8. Hepatitis was scored as number of foci per square centimeter of H&E-stained liver sections, counted by two blinded individuals. **B:** Numbers of influenza-specific D^b/NP tetramer$^+$ $CD8^+$ T cells in the lung airway (BAL) are plotted after primary influenza infection (black circle) of naïve B6 mice or secondary infection (gray square) of HK/x31-immune B6 mice, as in **A**. Mean numbers of NP^+CD8^+ T cells in the BAL were calculated by multiplying flow cytometric percentages by cell counts for pooled BAL samples and then correcting to represent the expected value from a single mouse. **C:** Influenza-specific D^b/NP tetramer$^+$ $CD8^+$ T cells in the liver at the peak of the immune response primary infection (day 8). Percentage of lymphocytes that are $CD8^+$ NP-tetramer$^+$ is shown next to region. **D:** During the peak of secondary infection, a higher proportion of influenza-specific D^b/NP tetramer$^+$ $CD8^+$ T cells localize to the liver. Percentage of lymphocytes that are $CD8^+$ NP-tetramer$^+$ on day 7 is shown next to the region.

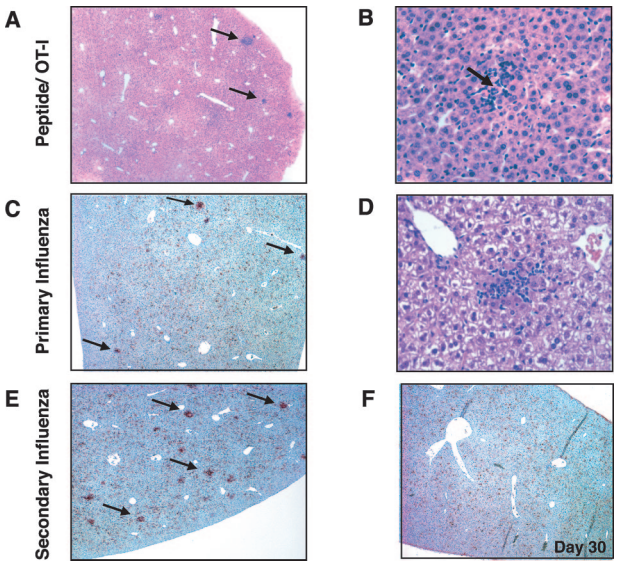

Figure 3. Histological evidence of hepatitis in peptide-treated and influenza-infected mice. **A–F:** Formalin-fixed liver sections stained with hematoxylin and eosin (**A**, **B**, and **D**) or anti-F4/80 (**C**, **E**, and **F**); **arrows** indicate inflammatory foci. **A:** C57BL/6 mice received 5×10^6 adoptively transferred OT-I $CD8^+$ T cells followed by activation with intraperitoneal injection of 25 nmol/L SIINFEKL peptide in saline on days 0, 1, and 2; sections taken on day 5 (Original magnification, ×20). **B:** High magnification (×400) focus after T-cell activation by peptide; **arrow** indicates Councilman body. **C:** C57BL/6 livers harvested on day 8 after primary intranasal infection with 10^5 EID_{50} influenza A/HKx31 (Original magnification, ×20). **D:** High magnification focus after primary influenza infection (×400). **E:** Hepatic sections 6 days after secondary influenza A/PR/8 infection (Original magnification, ×20). **F:** Liver section 30 days after primary influenza infection (Original magnification, ×20).

mice probably reflects the fact that all mice underwent a uniform primary infection without the possibility of a prior cross-reactive infection, unlike non-naïve human subjects.

Hepatitis Is Not Related to Virus Replication in the Liver

A plausible explanation for liver damage is that it is a consequence of co-infection of the liver itself during the course of respiratory infection. During human influenza infection, the occurrence of viremia is not well documented. To evaluate the extent of intrahepatic infection with influenza virus, liver and lung samples from infected mice were subjected to quantitative real-time RT-PCR to detect the virus genome. The results indicated large copy numbers of influenza virus genome in day-4 lungs (Figure 1E), but the livers did not differ from uninfected controls. The RT-PCR-based assay shows that the occurrence and quantity of infectious virus in the livers were not consistent with the high incidence (100%) and magnitude of the hepatitis or with the reproducible incidence of significant proportions of intrahepatic influenza-specific T cells found in all infected animals (as quantified by flow cytometry; Figure 2). Inoculation of eggs with tissue samples from acutely infected animals also failed to show evidence of virus in the liver (data not shown). All evidence indicates that infection of the liver does not play a role in the pathology seen at that site.

Hepatitis Occurs during Clonal Expansion of $CD8^+$ T Cells

In a model using transgenic $CD8^+$ T cells (OT-I), injection of their cognate peptide induces antigen-specific T-cell activation and proliferation. This is followed by intrahepatic trapping of these activated T cells, with evidence of hepatic inflammation and damage.[5,21,22] Similarly, influenza-specific $CD8^+$ T cells are detectable in the liver after influenza infection in the lung.[6] Because the elevated liver aminotransaminase levels indicated hepatic damage in this respiratory infection model, we compared the liver pathology to the established peptide model. Naïve $CD8^+$ T cells were stimulated in B6 mice either by injection of SIINFEKL peptide (Figure 3, A and B) after adoptive transfer of $CD8^+$OT-I T cells from transgenic donors or by respiratory infection with influenza x31 (Figure 3, C and D). Intrahepatic focal accumulations characterized by dense aggregates of mononuclear cells were visible in both the peptide and infection models (Figure 3, A–D). During x31 infection, the peak lymphocyte response in the lung was accompanied by formation of discrete foci containing eosinophilic Councilman bodies (the histological manifestation of apoptotic hepatocytes; Figure 3D) throughout the lobes of the liver but without any distinct zonal distribution (Figure 3C). Hepatitis resolved within 30 days of infection (Figure 3F). Subsequent hetero-subtypic challenge with influenza PR8 produced greater numbers of hepatitic foci (Figure 3E)

than primary infection. Accumulations during secondary infection were otherwise indistinguishable in size and content from those in either primary-infected mice or OT-I/peptide recipients.

The Antiviral CD8$^+$ T-Cell Response Drives the Development of Hepatitis

Preliminary histology indicated significantly greater hepatitis on secondary challenge of a memory population (Figure 3, C and E). Because the memory CD8$^+$ T-cell response is significantly more robust than the primary response, this suggested to us that the magnitude of damage may correlate with the size of the CD8$^+$ T-cell response to infection, rather than with the quantity of virus. To test the hypothesis that the presence of T cells directly correlated with damage, we quantified both the hepatitis and the CD8$^+$ T-cell response to the virus over time. A change in number or kinetics of activated CD8$^+$ T cells in the system was predicted to translate into a similar change in hepatic damage.

On day 6 of a primary infection, inflammatory foci became visible in the livers (Figure 2A, circles), and serum transaminase levels were elevated above normal (Figure 1, C and D). The number of hepatitic foci and the level of serum enzymes both peaked on day 8 (Figures 2 and 1), coinciding with the peak of influenza-specific CD8$^+$ T-cell numbers in the lung airways (Figure 2B, circles), as determined by flow cytometric analysis of D^b/NP-tetramer$^+$ T cells in the BAL. In addition, the decline in hepatitis paralleled the disappearance of T cells from the lung, with a slight delay in resolution of hepatic inflammation. This suggests that the magnitude of hepatitis is driven by the size of the CD8$^+$ T-cell immune response.

We next varied the magnitude and timing of the CD8$^+$ T-cell response. During a secondary PR8 infection of x31-immune animals, the CD8$^+$ T-cell response is both greater in magnitude and occurs earlier,[10,23–25] because of the expansion of memory T cells. Quantitative analysis of livers during the re-challenge revealed that foci began to develop on day 4 (Figure 2A, squares), after NP-specific cells were already detectable in the draining lymph node.[13] Focal damage was notably more severe, despite the more rapidly cleared secondary virus infection (not shown). There was a close correlation between virus-specific NP$^+$CD8$^+$ T-cell numbers in the lung airways (Figure 2B, squares) and maximum number of foci in the liver (Figure 2A), both of which increased about 2.5-fold compared with the primary infection, again supporting the view that hepatitis is driven by the CD8$^+$ response. In fact, significant numbers of influenza-specific T cells can be isolated from the liver at the peak of the immune response to either primary or secondary infection, as determined by NP-tetramer staining. On day 8 of primary infection, around 2.6% of liver lymphocytes are NP-tetramer$^+$ (Figure 2C), which translates to 1.5×10^5 antigen-specific CD8$^+$ T cells. During the peak response to secondary PR8 infection, the proportion of intrahepatic NP-tetramer$^+$ CD8$^+$ cells rises to 7% (Figure 2D), about 4×10^5 influenza NP-specific cells. In comparison, at the site of infection, the proportion of these cells in the BAL (not shown) increases from 6% during primary infection to 18% during secondary infection (2×10^5 NP-specific T cells in the BAL during PR8 infection; Figure 2B). Thus, liver injury correlates with kinetics and number of influenza-specific T cells generated during the immune response as well as with their presence in the liver.

Virus-Primed CD8$^+$ T Cells Preferentially Localize to the Foci

The development of hepatitis during acute infection, when there are large numbers of circulating activated CD8$^+$ T cells, suggested that the pathology was a consequence of the characteristic ability of the liver to selectively trap activated CD8$^+$ T cells.[26] If the influenza-specific CD8$^+$ T cells are to nucleate focus formation, they should be detectable within the foci. To determine whether virus-primed cells were actually present in foci, Thy1.1$^+$CD8$^+$ OT-I T cells were adoptively transferred into naïve Thy1.2$^+$ B6 recipients. The mice were then infected with recombinant influenza A/WSN-OVA$_I$ in which the OVA epitope SIINFEKL acts as a surrogate viral antigen,[15] causing expansion of the transferred OT-I T cells. After activation by the influenza infection, the transgenic OT-I CD8$^+$ T cells accumulated in the liver as seen before and made up 8% of the total liver CD8$^+$ T cells at the peak of the immune response (Figure 4A), whereas livers from uninfected transfer recipients contained 0.2% donor-derived OT-I cells. Immunohistological sections revealed that these virus-specific OT-I cells preferentially localized to the inflammatory foci (Figure 4B). Each focus contained a mixture of host Thy1.2$^+$ cells (the majority) and donor Thy1.1$^+$ cells. In contrast, uninfected animals that had received OT-I cells did not have intrahepatic focal lesions (although rare donor cells were scattered diffusely throughout the liver), further indicating that CD8$^+$ T-cell priming was necessary for focus formation.

The potential participation of other cell types in damage induction was still unclear. Some focus-associated cells stained positive for CD4 (not shown), although these may be T cells, NK T cells, or macrophages, each of which are capable of CD4 expression. Additional immunohistochemical staining of focal infiltrates revealed that significant numbers of F4/80$^+$ Kupffer cells were in close contact with the CD3$^+$ T cells (Figure 4C). Apoptosis within the foci was assessed by TUNEL staining, which revealed that CD3$^+$ T cells (Figure 4D) and non-T cells were undergoing apoptosis. Together, Councilman bodies and TUNEL$^+$ CD3$^-$ F4/80$^-$ cells indicated bona fide hepatocyte damage. Dying TUNEL$^+$ cells were entwined with F4/80$^+$ Kupffer cells (Figure 4, E and F), suggesting that the interaction between these cells regulates focus formation and possibly subsequent T-cell and hepatocyte death.

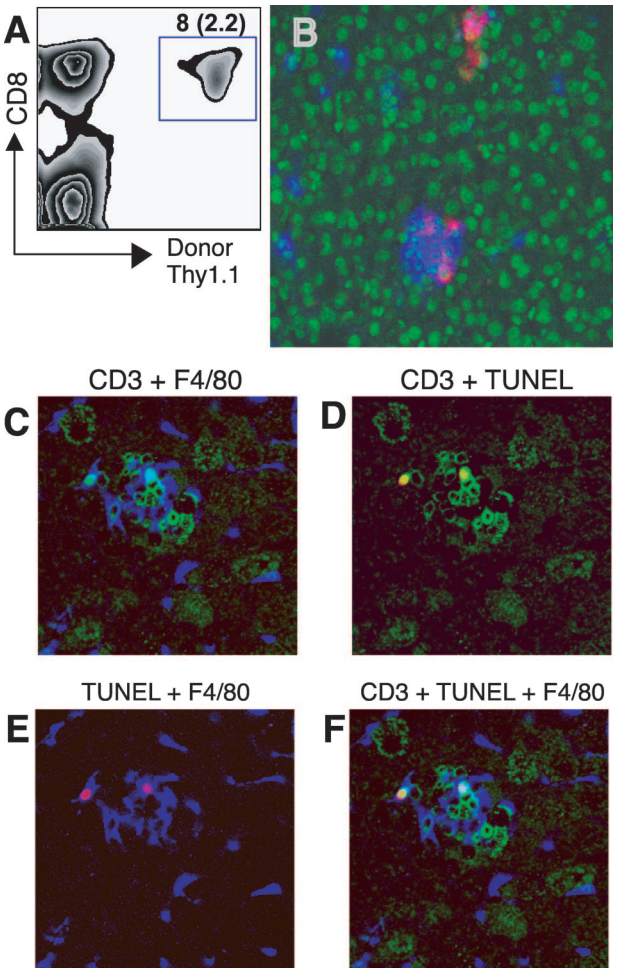

Figure 4. Antigen-specific $CD8^+$ T cells are a necessary component of hepatitic foci. **A:** Naïve $Thy1.1^+$ $CD8^+$ OT-I T cells (5×10^6) were transferred into C57BL/6 ($Thy1.2^+$) mice that were subsequently infected with 10^3 EID_{50} of influenza WSN-OVA_I. On day 9 after infection, intrahepatic lymphocytes were stained with antibodies against CD8, Thy1.1, and Thy1.2 for flow cytometric analysis; plot is gated on live lymphocytes. Numbers indicate the percentage of CD8s (in parentheses, percentage of total lymphocytes) staining with indicated markers. **B:** Identification of virus-specific $CD8^+$ T cells in the foci of infected mice. Frozen sections of livers from **A** were stained with anti-Thy1.1 showing donor OT-I cells (red), anti-Thy1.2 showing host lymphocytes (blue), and nuclear counterstain Sytox Green. **C–F:** Immunohistochemical analysis of individual foci during influenza infection in frozen liver sections stained with combinations of anti-CD3-FITC (green), TUNEL reagent (red), and F4/80-APC (blue), as indicated (Original magnification, ×400). Cellular makeup of foci includes numerous $CD8^+$ T cells and Kupffer cells.

Kupffer Cells Are Necessary for Focus Formation and Hepatocyte Damage, but Not for $CD8^+$ T-Cell Accumulation

The visualization of Kupffer cells within foci and in close association with apoptotic cells raised the question of their role in the pathology. Their presence could signify their intimate involvement in focus formation and hepatic damage, or they may merely be attracted to these pockets of damage after the fact, as a function of their scavenging capabilities. To distinguish between these two possibilities, Kupffer cells were selectively depleted during the course of a secondary PR8 infection. Forty-eight hours after being infected, mice were intravenously injected with clodronate-containing liposomes,[14] which are preferentially endocytosed by Kupffer cells and induce their apoptosis.

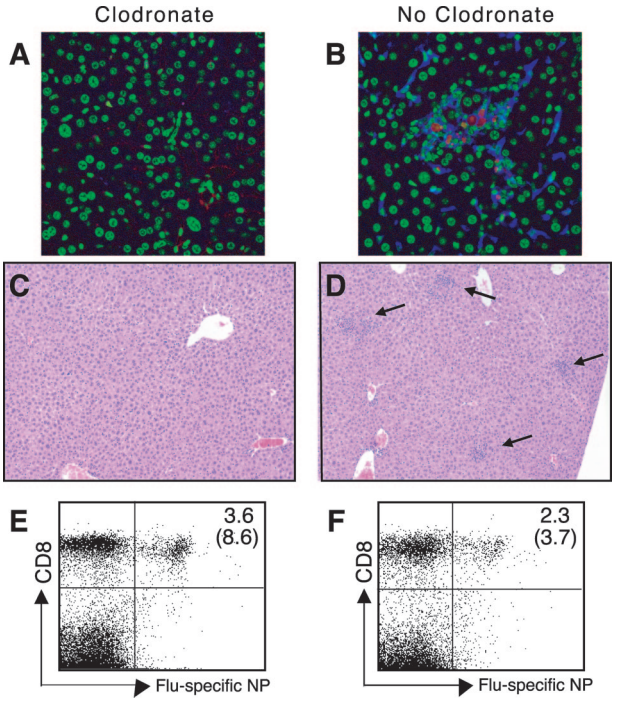

Figure 5. Kupffer cells are required for influenza-induced collateral liver damage. On day 2 of secondary infection with influenza A/PR8, mice received 200 μl of clodronate liposomes intraperitoneally. On day 5, livers were sectioned and stained. **A** and **B:** Frozen sections were stained with anti-F4/80 (Kupffer cells, blue), TUNEL (red), and nuclear counterstain Sytox Green (×400). Kupffer cells were absent after administration of clodronate (**A**) compared with PBS controls **B**. **C** and **D:** H&E-stained sections (×40) from clodronate-treated (**C**) and PBS control mice (**D**) show that focus formation was abrogated by Kupffer cell depletion. **E** and **F:** Flow cytometric analysis of virus NP-specific $CD8^+$ T cells after clodronate (**E**) or PBS (**F**) injection. Numbers indicate percentage of virus-specific CD8s in livers; lower numbers in parentheses indicate percentage in lung airways.

At the peak of the secondary immune response in the lung (day 5), immunohistological staining of clodronate-treated livers revealed complete ablation of Kupffer cells (Figure 5A), in comparison with mice that received only PBS (Figure 5B). Significantly, these Kupffer cell-depleted livers were dramatically protected from influenza-associated focus formation (Figure 5C; mean, 2.0 foci/cm^2), and Councilman bodies were absent. In contrast, infected mice with an intact Kupffer cell population (PBS control) exhibited robust hepatitis (Figure 5D; mean, 114.5 foci/cm^2) and characteristic focus-associated apoptosis (Figure 5B) with abundant Councilman bodies (51 per cm^2). The requirement for both $CD8^+$ T cells and Kupffer cells and the demonstration of specific $CD8^+$ T cells within foci indicate that hepatitis is a direct consequence of expansion and intrahepatic trapping of activated $CD8^+$ T cells that interact with Kupffer cells to damage hepatocytes.

Although formation of foci depended on the presence of Kupffer cells, the intrahepatic accumulation of $CD8^+$ T cells did not. NP-specific $CD8^+$ T-cell populations could be isolated from the livers of both clodronate-treated (Figure 5E) and control (Figure 5F) groups. In fact, influ-

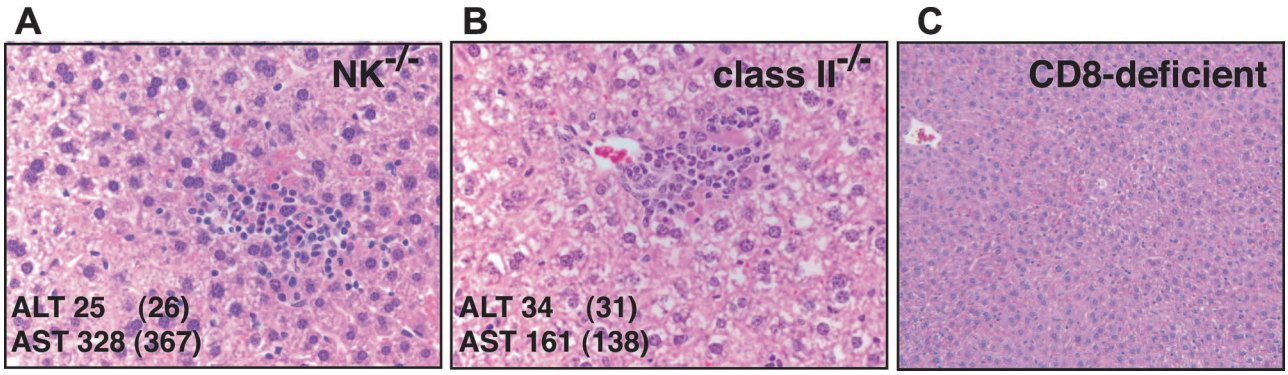

Figure 6. Neither NK nor $CD4^+$ T cells are required for the induction of CD8-dependent collateral liver damage. **A:** NK-deficient mice were infected with 10^5 EID_{50} influenza A/HK/x31, and day-8 liver sections were H&E-stained, revealing numerous foci and Councilman bodies (×400). Mean serum transaminase levels are indicated (in parentheses, control group transaminases). **B:** Focus typical of H&E-stained liver sections from MHC class II-deficient mice, infected 9 days prior with 10^5 EID_{50} influenza A/HK/x31 (×400). Mean serum transaminase levels are indicated (in parentheses, control group transaminases). **C:** TCR $C\alpha^{-/-}$ mice (with no endogenous T-cell response) received 2×10^7 DO11.10 lymphocytes ($CD4^+$ T cells specific for $OVA_{323–339}$) intravenously and were subsequently infected with 500 pfu of influenza WSN-$OVA_{323–339}$. Day-9 livers were harvested, and sections were H&E-stained. These mice contained influenza-activated $CD4^+$ T cells in the absence of $CD8^+$ T cells, and correspondingly, hepatitis was not visible histologically (×100).

enza-specific cells in both livers and airways of treated mice accumulated in higher proportion and greater number than in PBS controls, indicating that antigen-specific activation was not impaired by clodronate treatment. It is therefore likely that $CD8^+$ T cells become trapped in the liver by a largely Kupffer cell-independent mechanism, possibly via ICAM-1 or VCAM-1.[27] There was a subtle increase in the number of specific $CD8^+$ T cells in clodronate-treated livers, and this may reflect the interruption of some immune regulatory function in the absence of the macrophage population of the liver. Kupffer cell depletion during viral infection thus clearly demonstrates that liver damage and focus formation in influenza is strictly dependent on the presence of Kupffer cells and that instead of promoting expansion or accumulation of the $CD8^+$ T cells, they may participate in regulating $CD8^+$ T-cell numbers.

Focal Lesions Are Not Dependent on the Presence of NK Cells or $CD4^+$ T Cells

To understand if other liver resident cell types contributed to focus formation, NK-deficient mice were infected with influenza. On day 8 of infection, NK-deficient and wild-type B6 livers exhibited similar numbers of inflammatory foci with comparable focal patterns containing Councilman bodies (Figure 6A). In addition, serum transaminase levels were equivalently elevated in both groups (mean ± SE: *ALT* in control = 26 ± 4.8, in NK-deficient = 25 ± 5.1; *AST* in control = 367 ± 138, in NK deficient = 328 ± 98). $CD8^+$ T-cell infiltration, determined by flow cytometry, was comparable in both groups (data not shown). Collectively, these data indicate that NK cells were not required for either $CD8^+$ T-cell accumulation or focus formation.

Infection of MHC class II-deficient mice (class $II^{-/-}$) lacking $CD4^+$ T cells also resulted in hepatitis (Figure 6B) and elevated transaminase levels as in control mice (mean ± SE: *ALT* in control = 31 ± 1.5, in MHC class $II^{-/-}$ = 34 ± 4.7; *AST* in control = 138 ± 16, in MHC class $II^{-/-}$ = 161 ± 58). This indicates that $CD4^+$ T cells are unlikely to be necessary for influenza-induced hepatitis, a conclusion that is further supported by an experiment in mice containing $CD4^+$ T cells but not $CD8^+$ T cells. Transgenic $CD4^+$ DO11.10 T cells, which are specific for the $OVA_{323–339}$ peptide, were transferred into C-α-deficient mice (devoid of endogenous T-cell receptors) and then stimulated with influenza expressing $OVA_{323–339}$. This adoptive transfer generated a mouse lacking $CD8^+$ T cells but containing antigen-specific $CD4^+$ T cells capable of recognizing the influenza virus. In this system, no foci were detectable histologically (Figure 6C), despite evidence of $CD4^+$ T-cell activation[16] and lymphocytic infiltrate into the lung during viral clearance (data not shown).

Discussion

Our results extend previous findings of liver damage after systemic immune responses[5,28] to an extrahepatic antiviral response. We provide evidence that T-cell-induced collateral liver damage occurs during influenza infections; this process may manifest during other human respiratory infections or be exacerbated when combined with environmental or pharmacological hepato-toxicants. It is possible that the accumulation of pathogen-specific $CD8^+$ T cells resulting in liver damage is a feature of responses to many nonhepatotrophic infections and could explain the idiopathic hepatitis noted in numerous acute infections such as measles[29–31] and SARS.[32–34]

In humans infected with influenza, abnormal liver function tests have been noted[1–3] but without formal investigation or description of influenza-induced hepatitis. Although only 20% of humans infected with influenza exhibited elevated liver aminotransaminase levels in the material we examined, this can be attributed to variability in pre-existing levels of immunity from past influenza infections and to genetic differences among subjects. Thus, different magnitudes of $CD8^+$ T-cell immune responses among human subjects could result in the disparities we see in liver damage. There are a number of factors that dictate the severity of infection and the sub-

sequent immune response, including pre-existing antibodies, ability to present antigens, and variances in cytokine milieu. The data suggest that in humans, pre-existing influenza immunity may modify the incidence of hepatitis and liver damage in two distinct ways. One is that high levels of cross-reactive neutralizing antibodies may reduce the magnitude and incidence of successful respiratory infection and thereby reduce liver damage. Alternatively, high frequencies of cross-reactive $CD8^+$ T cells may produce a large $CD8^+$ T-cell response to the challenge infection and drive more severe liver injury. Thus, those patients who mounted the most robust $CD8^+$ T-cell responses to the infection, either as a result of insufficient anti-influenza antibody levels to limit infection or due to the presence of cross-reactive $CD8^+$ T-cell memory, would be the ones to exhibit liver injury. Therefore, the next phase of investigation is to measure antibody levels and frequencies of influenza-specific $CD8^+$ T cells in the blood of patients exhibiting elevated ALTs and ASTs. Unfortunately, the variable nature of immunity to influenza in humans would be difficult to replicate in our animal models.

During infection, virus-specific $CD8^+$ T cells undergo massive expansion.[10,23,35,36] A hallmark of adaptive immune responses and memory is that during secondary challenge, both magnitude and speed of the antiviral response is increased dramatically. In mice, primary infection with influenza A/HK/x31 followed by secondary challenge with influenza A/PR/8 provides an ideal system for studying consequences of anti-viral $CD8^+$ T-cell expansion, because these two viruses share an immunodominant internal $CD8^+$ T-cell epitope in the NP protein but generate nonoverlapping antibody responses.

The finding that the extent of hepatitis in primary and secondary infections is mirrored by the magnitude of the cellular response rather than viral infection implicates $CD8^+$ T cells in collateral liver damage. The data do not support the possibility that T-cell accumulation in the liver was in response to intrahepatic viral infection. In fact, previous studies have established that MHC expression by hepatocytes is not required to trap T cells,[26,28] although expression of MHC on bone marrow-derived cells in the liver promotes T-cell apoptosis.[37] Instead, accumulation of activated $CD8^+$ T cells appears to be driven by the expression of ICAM-1 and VCAM-1 in hepatic sinusoids and LFA-1 and VLA-4 on activated $CD8^+$ T cells.[26,27] It is interesting that the infiltrates are distributed through the liver parenchyma rather than in the periportal areas. This pattern is, however, consistent with the model we have proposed in which the high expression of integrin adhesion molecules, particularly VCAM-1, on the liver sinusoidal endothelium is responsible for the "passive" (ie, the antigen-independent) sequestration of activated $CD8^+$ T cells.[27] It is likely that the trapping of different subsets of T cells in the liver depends on different molecules; thus vascular adhesion protein-1 was recently shown to promote the recruitment of T helper-2, but not T helper-1, $CD4^+$ T cells to the liver during Con A-induced hepatitis.[38] However, the Con A model is difficult to interpret because Con A is a TCR ligand, but it is not clear whether this model simulates antigen-dependent "active" T-cell sequestration[27] or the antigen-independent mechanism that we envisage in the case of influenza.

The presence of virus-specific cells in the foci argues that the pathology is a direct consequence of the immune response. When antigen-specific Thy-1.1^+ OT-I T cells were responding to $WSN\text{-}OVA_I$ influenza,[15] most of the foci contained donor Thy1.1^+ cells (Figure 5D), although they were only a minority of the total T cells in each focus, whereas the rest were of host origin. Of note, the host Thy1.2^+ response was occurring simultaneously against both the OVA epitope and non-OVA-specific influenza antigens. Whether all $CD8^+$ T cells within such foci are specific for the virus is not yet clear. Nonetheless, the presence of known antigen-specific T cells in most foci, along with the requirement for activated $CD8^+$ T cells for hepatitis, suggests that these cells are involved in initiating focus formation.

Foci also contained damaged hepatocytes and bone marrow-derived Kupffer cells, which may be required to induce the apoptosis of T cells.[39] The significance of the Kupffer cells in influenza-associated hepatitis is not fully understood. One possibility is that, despite the lack of infectious virus or viral RNA in the liver, Kupffer cells are presenting influenza antigens that reached the liver by an undefined mechanism. However, we do not think this is likely to be an important mechanism in T-cell recruitment because in secondary influenza, the infection is of shorter duration and the virus load is lower because of rapid immune control, yet the liver lesions are more florid, and the hepatocellular injury is greater. This identifies the T cells rather than antigen as the prime mover in influenza-associated hepatitis. As for the significance of Kupffer cells, we envisage that they are recruited by already activated T cells that become sequestered by the antigen-independent mechanisms but then act as a site for the elimination of these cells, as already proposed based on experiments in an antigen-dependent model.[37]

The observation that depletion of Kupffer cells leads to an increase in the number of influenza-specific $CD8^+$ T cells in both the lung and liver is consistent with the idea that the liver regulates the supply of $CD8^+$ T cells in the system, perhaps by influencing their sequestration or actively promoting deletion. Kupffer cells are not casual observers of influenza-induced damage; rather, their presence in an activated state provokes the convergence of cells into foci and the generation of Councilman bodies. The mechanism promoting this activation of Kupffer cells remains in question. One possibility is that activated cytolytic $CD8^+$ T cells induce an activation state in Kupffer cells. Interleukin-12, tumor necrosis factor-α (TNF-α), and interferon-γ have been implicated in Kupffer cell activation and hepatic damage in other experimental models.[28,40] Specifically, Kupffer cell-derived TNF-α is known to be injurious to hepatocytes in several T-cell-dependent acute liver damage models.[41] Kupffer cell phagocytosis of apoptotic bodies has also been shown to result in the elaboration of TNF-α and FasL, leading to hepatocyte injury.[42] One previous report using peptide-induced activation of T cells in OT-I transgenic mice showed that the absence of FasL inhibited hepatocyte

damage, although those cells on which expression of FasL was important were not identified.[43] Given the critical role of Kupffer cells in the induction of collateral liver damage, we predict that Kupffer cells are the cellular source of the FasL in that model.

The lack of involvement of NK cells in collateral liver damage is noteworthy because NK cells are important in several other models of liver injury.[44,45] Thus, depletion of NK cells ameliorates T-cell-dependent liver damage induced by the mitogen *Pseudomonas aeruginosa* exotoxin A[46] and $CD8^+$ T-cell-dependent injury during adenovirus infection[47]; furthermore, it compromises recruitment of defensive $CD8^+$ T cells in murine CMV infection.[45] The common denominator of all of these experimental models is the presence of the T-cell stimulus (either *P. aeruginosa* exotoxin A or virus) in the liver. Despite the similarity of the histological lesions, the mechanism of collateral liver damage in the absence of intrahepatic antigen appears to be totally different from the recruitment of a local antiviral response, and this is illustrated by the lack of a role for NK cells.

The current data do not exclude that in the presence of CD8 and Kupffer cells, other cells may localize within the foci and participate in a damage cascade. In fact, within foci of intact animals, a few cells did stain with anti-CD4 and anti-NK1.1 antibodies (not shown). However, the evidence presented here indicates that any other cells that may become involved must do so downstream of an interaction between Kupffer cells and virus-specific $CD8^+$ T cells.

The liver sinusiodal endothelial cells constitutively express adhesion molecules such as ICAM-1 and VCAM, which may result from chronic exposure to intestinal endotoxin entering via the portal vein[48] but are not normally highly expressed on the endothelial cells of uninflammed capillary beds elsewhere. The interaction between these "inflammatory" adhesion molecules and LFA-1 expressed on activated $CD8^+$ T cells leads to intrahepatic trapping and Kupffer cell-mediated killing of at least some of the accumulated $CD8^+$ T cells. This phenomenon could merely be the functional consequence of the relative stickiness of the liver for activated $CD8^+$ T cells. Alternatively, there may be an advantage to intrahepatic trapping of activated lymphocytes during immune responses. The immune system is known to respond to respiratory infections with considerable excess of activated lymphocytes above those needed for viral clearance.[13,36] This apparent inefficiency may reflect programming of the response independent of antigenic load.[49] Although intrahepatic trapping of potential effector T cells may result in a dampened cellular response and delayed viral clearance from extrahepatic sites, it could serve to modulate the $CD8^+$ response and limit immunopathology in other organs. Because the liver is able to regenerate after parenchymal injury,[50] sustaining collateral damage may be a calculated sacrifice worth the production of a robust lymphocyte response.

The significance of collateral liver damage in human disease remains to be evaluated. During acute influenza infection in two small samples of healthy volunteers, the liver injury was always benign and transient. However, it is not clear that this would be the case in immunodeficient individuals or in anyone with pre-existing liver disease. Different strains of influenza may also vary in their capacity to cause collateral liver damage, and this is a concern as new strains arise by natural recombination. We also do not know whether chronic $CD8^+$ T-cell activation at an extrahepatic site could cause chronic collateral liver damage. The mechanism described here would be a candidate in the case of inflammatory hepatitides of unknown origin.

Acknowledgments

We thank Dr. Fred Hayden and the University of Virginia for contributing to the influenza studies in humans.

References

1. Wirth S: ["Accompanying hepatitis" in viral infections]. Dtsch Med Wochenschr 1995, 120:461
2. Yuen KY, Chan PK, Peiris M, Tsang DN, Que TL, Shortridge KF, Cheung PT, To WK, Ho ET, Sung R, Cheng AF: Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet 1998, 351:467–471
3. Chan PK: Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. Clin Infect Dis 2002, 34(Suppl 2):S58–S64
4. Huang L, Soldevila G, Leeker M, Flavell R, Crispe IN: The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. Immunity 1994, 1:741–749
5. Russell JQ, Morrissette GJ, Weidner M, Vyas C, Aleman-Hoey D, Budd RC: Liver damage preferentially results from CD8(+) T cells triggered by high affinity peptide antigens. J Exp Med 1998, 188:1147–1157
6. Belz GT, Altman JD, Doherty PC: Characteristics of virus-specific CD8(+) T cells in the liver during the control and resolution phases of influenza pneumonia. Proc Natl Acad Sci USA 1998, 95:13812–13817
7. Eichelberger MC, Wang ML, Allan W, Webster RG, Doherty PC: Influenza virus RNA in the lung and lymphoid tissue of immunologically intact and CD4-depleted mice. J Gen Virol 1991, 72:1695–1698
8. Hamilton-Easton A, Eichelberger M: Virus-specific antigen presentation by different subsets of cells from lung and mediastinal lymph node tissues of influenza virus-infected mice. J Virol 1995, 69:6359–6366
9. Belz GT, Xie W, Altman JD, Doherty PC: A previously unrecognized H-2D(b)-restricted peptide prominent in the primary influenza A virus-specific CD8(+) T-cell response is much less apparent following secondary challenge. J Virol 2000, 74:3486–3493
10. Flynn KJ, Belz GT, Altman JD, Ahmed R, Woodland DL, Doherty PC: Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. Immunity 1998, 8:683–691
11. Doherty PC, Effros RB, Bennink J: Heterogeneity of the cytotoxic response of thymus-derived lymphocytes after immunization with influenza viruses. Proc Natl Acad Sci USA 1977, 74:1209–1213
12. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM: In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci USA 2000, 97:2731–2736
13. Allan W, Tabi Z, Cleary A, Doherty PC: Cellular events in the lymph node and lung of mice with influenza: consequences of depleting CD4+ T cells. J Immunol 1990, 144:3980–3986
14. Van Rooijen N, Sanders A: Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J Immunol Methods 1994, 174:83–93
15. Topham DJ, Castrucci MR, Wingo FS, Belz GT, Doherty PC: The role of antigen in the localization of naive, acutely activated, and memory CD8(+) T cells to the lung during influenza pneumonia. J Immunol 2001, 167:6983–6990
16. Chapman TJ, Castrucci MR, Padrick RC, Bradley LM, Topham DJ:

Antigen-specific and non-specific CD4(+) T cell recruitment and proliferation during influenza infection. Virology 2005, 340:296–306
17. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM: Phenotypic analysis of antigen-specific T lymphocytes. Science 1996, 274:94–96
18. Pierce RH, Vail ME, Ralph L, Campbell JS, Fausto N: Bcl-2 expression inhibits liver carcinogenesis and delays the development of proliferating foci. Am J Pathol 2002, 160:1555–1560
19. Pierce RH, Campbell JS, Stephenson AB, Franklin CC, Chaisson M, Poot M, Kavanagh TJ, Rabinovitch PS, Fausto N: Disruption of redox homeostasis in tumor necrosis factor-induced apoptosis in a murine hepatocyte cell line. Am J Pathol 2000, 157:221–236
20. Hou S, Hyland L, Ryan KW, Portner A, Doherty PC: Virus-specific CD8+ T-cell memory determined by clonal burst size. Nature 1994, 369:652–654
21. Crispe IN, Mehal WZ: Strange brew: T cells in the liver. Immunol Today 1996, 17:522–525
22. Crispe IN, Dao T, Klugewitz K, Mehal WZ, Metz DP: The liver as a site of T-cell apoptosis: graveyard, or killing field? Immunol Rev 2000, 174:47–62
23. Flynn KJ, Riberdy JM, Christensen JP, Altman JD, Doherty PC: In vivo proliferation of naive and memory influenza-specific CD8(+) T cells. Proc Natl Acad Sci USA 1999, 96:8597–8602
24. Cauley LS, Cookenham T, Hogan RJ, Crowe SR, Woodland DL: Renewal of peripheral CD8+ memory T cells during secondary viral infection of antibody-sufficient mice. J Immunol 2003, 170:5597–5606
25. Hogan RJ, Cauley LS, Ely KH, Cookenham T, Roberts AD, Brennan JW, Monard S, Woodland DL: Long-term maintenance of virus-specific effector memory CD8+ T cells in the lung airways depends on proliferation. J Immunol 2002, 169:4976–4981
26. Mehal WZ, Juedes AE, Crispe IN: Selective retention of activated CD8+ T cells by the normal liver. J Immunol 1999, 163:3202–3210
27. John B, Crispe IN: Passive and active mechanisms trap activated CD8+ T cells in the liver. J Immunol 2004, 172:5222–5229
28. Bowen DG, Warren A, Davis T, Hoffmann MW, McCaughan GW, De St Groth BF, Bertolino P: Cytokine-dependent bystander hepatitis due to intrahepatic murine CD8 T-cell activation by bone marrow-derived cells. Gastroenterology 2002, 123:1252–1264
29. Satoh A, Kobayashi H, Yoshida T, Tanaka A, Kawajiri T, Oki Y, Kasugai K, Tonai M, Satoh K, Nitta M: Clinicopathological study on liver dysfunction in measles. Intern Med 1999, 38:454–457
30. Shalev-Zimels H, Weizman Z, Lotan C, Gavish D, Ackerman Z, Morag A: Extent of measles hepatitis in various ages. Hepatology 1988, 8:1138–1139
31. Khatib R, Siddique M, Abbass M: Measles associated hepatobiliary disease: an overview. Infection 1993, 21:112–114
32. Chan HL, Kwan AC, To KF, Lai ST, Chan PK, Leung WK, Lee N, Wu A, Sung JJ: Clinical significance of hepatic derangement in severe acute respiratory syndrome. World J Gastroenterol 2005, 11:2148–2153
33. Hsiao CH, Chang MF, Hsueh PR, Su IJ: Immunohistochemical study of severe acute respiratory syndrome-associated coronavirus in tissue sections of patients. J Formos Med Assoc 2005, 104:150–156
34. Shi X, Gong E, Gao D, Zhang B, Zheng J, Gao Z, Zhong Y, Zou W, Wu B, Fang W, Liao S, Wang S, Xie Z, Lu M, Hou L, Zhong H, Shao H, Li N, Liu C, Pei F, Yang J, Wang Y, Han Z, Zhang Q, You J, Zhu X, Gu J: Severe acute respiratory syndrome associated coronavirus is detected in intestinal tissues of fatal cases. Am J Gastroenterol 2005, 100:169–176
35. Murali-Krishna K, Altman JD, Suresh M, Sourdive DJ, Zajac AJ, Miller JD, Slansky J, Ahmed R: Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 1998, 8:177–187
36. Tripp RA, Hou S, McMickle A, Houston J, Doherty PC: Recruitment and proliferation of CD8+ T cells in respiratory virus infections. J Immunol 1995, 154:6013–6021
37. Mehal WZ, Azzaroli F, Crispe IN: Antigen presentation by liver cells controls intrahepatic T cell trapping, whereas bone marrow-derived cells preferentially promote intrahepatic T cell apoptosis. J Immunol 2001, 167:667–673
38. Bonder CS, Norman MU, Swain MG, Zbytnuik LD, Yamanouchi J, Santamaria P, Ajuebor M, Salmi M, Jalkanen S, Kubes P: Rules of recruitment for Th1 and Th2 lymphocytes in inflamed liver: a role for alpha-4 integrin and vascular adhesion protein-1. Immunity 2005, 23:153–163
39. Sun Z, Wada T, Uchikura K, Ceppa E, Klein AS: Role of Fas/Fasl in Kupffer cell-dependent deletion of alloantigen activated T cells following liver transplantation. Transplant Proc 2001, 33:279–282
40. Tiegs G, Gantner F: Immunotoxicology of T cell-dependent experimental liver injury. Exp Toxicol Pathol 1996, 48:471–476
41. Schumann J, Wolf D, Pahl A, Brune K, Papadopoulos T, van Rooijen N, Tiegs G: Importance of Kupffer cells for T-cell-dependent liver injury in mice. Am J Pathol 2000, 157:1671–1683
42. Canbay A, Feldstein AE, Higuchi H, Werneburg N, Grambihler A, Bronk SF, Gores GJ: Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. Hepatology 2003, 38:1188–1198
43. Kennedy NJ, Russell JQ, Michail N, Budd RC: Liver damage by infiltrating CD8+ T cells is Fas dependent. J Immunol 2001, 167:6654–6662
44. Dobashi H, Seki S, Habu Y, Ohkawa T, Takeshita S, Hiraide H, Sekine I: Activation of mouse liver natural killer cells and NK1.1(+) T cells by bacterial superantigen-primed Kupffer cells. Hepatology 1999, 30:430–436
45. Salazar-Mather TP, Orange JS, Biron CA: Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1alpha (MIP-1alpha)-dependent pathways. J Exp Med 1998, 187:1–14
46. Muhlen KA, Schumann J, Wittke F, Stenger S, Van Rooijen N, Van Kaer L, Tiegs G: NK cells, but not NKT cells, are involved in Pseudomonas aeruginosa exotoxin A-induced hepatotoxicity in mice. J Immunol 2004, 172:3034–3041
47. Liu ZX, Govindarajan S, Okamoto S, Dennert G: NK cells cause liver injury and facilitate the induction of T cell-mediated immunity to a viral liver infection. J Immunol 2000, 164:6480–6486
48. Nolan JP: The role of endotoxin in liver injury. Gastroenterology 1975, 69:1346–1356
49. Wong P, Pamer EG: Cutting edge: antigen-independent CD8 T cell proliferation. J Immunol 2001, 166:5864–5868
50. Fausto N: Liver regeneration. J Hepatol 2000, 32:19–31